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The serodiagnostic of listeriosis.

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with 6 Figures.

Discourse No. 2, Listeriosis, a symposium at Giessen, Germany, 27 - 28 June 1957, pp 20-33. Supplement 1 to Zentralblatt fur Veterinarmedizin, 1958.

Hitherto existing knowledge of the frequency and dissemination of human listeriosis is based on approximately 500 bacteriologically verified findings from almost all parts of the world. More than 400 of these pertinent cases were recognized within a period of barely 5 years. This circumstance is mainly due to decisive improvement in bacteriological diagnostic everywhere, and also to the fact that previously mistaken or wrongly classified pathogens today are recognized with increasing frequency.

During the last few years listeriosis has lost its character of a seemi ly rare human disease; and we can hardly doubt that cases of illness diagnos to date represent only a fraction of the total number of human infections wh really have occurred. It is usually the case with every seemingly "new" infectious disease that the virulent forms are recognized first, upon joint efforts by the clinician, the bacteriologist and the pathologist. This has occurred in connection with toxoplasmosis, poliomyelitis, histoplasmosis and coccidioidomycosis, but also with various bacterial infectious diseases. Further studies then showed regularly regarding almost all of these infectious diseases, that the overwhelming majority of cases may progress clinically with few symptoms and partly even devoid of symptoms. In dealing with a pathogen as widely and almost ubiquitously distributed as Listeria monocytogenes, which has been demonstrated so far sporadically and epizootically in more than 30 different wild and domestic animals, one must expect a high probability of contact even on the part of humans, indicating a larger quota of infections than is being determined at this time.

If the number of proven human infections nevertheless remains within surveyable bounds, this circumstance probably is strongly based on the special dispositional moments with which the familiar severe clinical manifestations are connected, of which the disposition of age is only one of many possibilities. In addition, the bacteriologic determination of the pathogen had been effected heretofore almost exclusively in cases with pronounced clinical symptoms only, often only after death. However, this verification has succeeded only in exceptional cases in connection with the less characteristic forms of listeriosis, e.g. during pregnancy, although it has been proved that the dangerous newborn listeriosis is acquired while still in the womb. The fact that benign forms of listeriosis, in addition to the feared virulent kinds, are not at all rare was supported among others by the findings of Nyfeldt in connection with monomucleosis-like diseases and accidental findings of other authors in asymptomatic cases (for summary see 32).

In view of the many unanswered questions regarding pathogen verification of subacute, chronic and asymptomatic listeric infections it was concomitant to utilize the immunity raction of the host as an auxiliary means in detecting the distribution and frequency of listeriosis, as well as for diagnosis of cases not perceptible by bacteriologic means. This method has proved itself overwhelmingly at this studium of other infectious diseases in the past decade, e.g. in connection with american systemic mycosis, spotted fever, spinal infantile paralysis, toxoplasmosis, etc.

Now that initial difficulties in the production of useful antigen preparations of listeria for the purpose of antibody determination have been overcome and methodical conditions for the routine testing of sera specimens are created (25, 32, 33, 35, 36 and others), we are today faced with the question of value and the practical significance of the seroreaction to listeriosis.

The answering of this question requires separate consideration of different aspects touching not only on the problem of listeriosis, but also the overall possibilities and limitations of serodiagnostic as such.

Attempts made heretofore to secure antibodies serologically that would react to listeria or partial components of listeria antigen proceeded from the premise that we were dealing with pathogen-specific methods of determination which deviate in this respect from numerous other serodiagnostic processes (cf. Herrmann, 1957, 13).

The utilization of pathogen-specific seroreactions to listeriosis is based first of all on the supposition that the body of the infected or immunized person reacts with a measurable production of antibody. As is well known, the experimental stimulation of specific listeric antibody in considerable quantities succeeds without great difficulty, be it after artificial infection or after intravenous instillation of killed antigen preparations.

On the other hand, reports concerning analogous action in the spontaneously infected large animal, especially the human, are rarely uniform and often even contradictory. It is difficult to assess the extent to which methodically conditioned causes are involved in reports of total absence of listeric antibody from serum specimens of pertinent cases. The claim has proved to be valid — and in consonance with some of our own observations — that the verification of complete antibody does not succeed in all cases of human listeriosis, especially so if short-lived acute infections are quickly beaten back and cured with chemotherapeutical and antibiotic agents. This fact has its parallels in numerous other infectious diseases and does not speak against the possible worth of serologic test methods in listeriosis. It should be pointed out, along with Potel (29a), that final pronouncements on such findings are justified only if the presence of incomplete antibody also has been excluded.

Besides, a sufficiently large number of observations made in Germany, USA, USSR and Canada show that in a larger share of bacteriologically verified cases of human listeriosis pathogen-specific antibody has appeared, at times in considerable amounts, when the infected person's organism has had enough time to cope with the antigen and was able to do this in the first place on account of age (8, 14, 22, 23, 25, 26, 28, 29 30, 32, 33, 37 and others). The last

mentioned is not the case with newborn and infants in the first few months of life, for instance (32).

Starting with the observation of a typical O and H titer curve in a case of listeric meningitis during pregnancy, diagnosed and successfully treated in 1951 jointly with Leineweber (37), we have during the past 6 years very seldom seen an absence of listeria-specific antibody in the sera of genuinely infected adults, just as noticed by Potel, Patocka et al., Girard and many others. An example from recent times is given by the serologic findings of a 65-year old woman suffering from a glandular form of listeriosis, whose serum revealed typical titer curves.

The specificity of this antibody can be proved resolutely by corresponding neutralization tests.

For instance, if sera with listeria type 1 O and H antibody are neutralized with the homologous O antigen, there remains after exhaustion of O agglutinin only type 1 H agglutinin, which can be absorbed by the similarly constructed type 4 H antigen. Conversely, after neutralization with type 4 OH antigen only type 1 O agglutination occurs in such sera.

The fact that specific antibody appears in the sera of persons ill with listeriosis and infected animals does not signify, however, that all antibodies reacting with listeria antigen must be pathogen-specific. This is shown by examples from many areas of serology whose enumeration would by far exceed the bounds of this presentation.

For this reason thorough examinations were conducted to answer the question so importance in practice, to which extent heterogenic antibody can influence seroreactions with listeriosis.

As far as is known today, cross reactions between listeria and gramnegative intestinal germs do not play an important role (Drew 6, present author 32, and others). Only Jaeger and Myers (15) report on cross precipitations between the K antigen of a Coli type and L. monocytogenes type 4 rabbit antiserum. Since hardly anything is known about L or K antibodies against Coli antigens in human sera, no practical conclusions can be reached for the time being. From own observations it appears certain that sera of persons with typhus and paratyphus as well as infants with infectious coli enteritis as a rule do not show an increased listeria titer, even if a copious amount of antibody is present against the respective pathogen. The same applies to the reverse.

Analogously, neither the animal experiment nor the testing of pertinent human sera reveal material serologic group relationships between pasteurella and listeria (32). The same applies to the pathogens of brucellosis and tularemia.

This circumstance seems of considerable significance for the practice of serologic differentiation of unexplained febrile aspects.

The conditions are much more complicated when listeria and other gram-positive germs are compared:

Thus we (34) found partial antigen associations between diverse strains of Streptococcus faecalis, serologic group D, and O-antigen or listeria polysaccharide, serotypes 1 and 4. These cross reactions were demonstrated in the agar gel precipitation test and even in a few human sera with a high titer of type 1. The cross-reacting receptors can be removed by corresponding neutralization tests with only a slight loss of titer for listeria antigen. The evaluation of enterococcal titers are made as difficult by the results as those of listeria. Additional test processes are indispensable, especially those dealing with heterologous agglutinin neutralization.

According to Sachse and Potel (cited after Sachse, 31) it seems that hemosensitins of other hemolyzing types of streptococci also will react with listeric sera. These findings deserve special attention and require further clarification, above all in view of the determination of increased listeric titers made frequently by us in connection with diseases of the upper air passages, in adenoiditis and rheumatoses (Lang and Seeliger, 17).

Starting with the demonstration of vigorous staphylococcal agglutination in the serum of a child probably ill with listeriosis and the report by Drews (6) on the isolation of staphylococcal polysaccharide extracts by L. monocytogenes type 3 antiserum in a precipitation test, the author in cooperation with Beeck and Sulzbacher (3, 35, 36) made comparative examinations of listeric and staphylococcal serology, utilizing agglutination, precipitation and complement fixation reactions. Initially the tests covered only thermostabile body substances or polysaccharides. The results reveal distinct group reactions between L. monocytogenes type 1 or 2 and S. aureus, and especially clearly between L. monocytogenes type 3 and staphylococci, while types 4a and 4b do not show any antigen relationship with S. aureus (cf. Table 1).

The testing of 1709 human sera resulted in titer distribution as listed in Table 2.

It is evident that a very close relationship exists between the reactions of listeria type 3 and S. aureus. In view of the great rarity of bacteriological determination of listeria type 3 in these parts, but also elsewhere, the relatively frequent seroreactions with precisely this type of antigen upon agglutination as well as complement fixation tests (CFT) must therefore evoke suspicions of unspecificity or heterologous reactions. This point was firmed up in neutralization tests, in which the type 3 titer often was caused to disappear by absorption with S. aureus antigen. For this reason we have for the time being desisted from any attempt to evaluate type 3 titers in the routine.

The situation seems different with listeria type 1. Positive agglutination titers and complement deflections in the presence of listeria type 1 antigen agree only in part of the cases — and this in about the same frequency as in listeria-negative sera — with S. aureus serotiters. This was supported by 3,000 additional serologic analyses. Even though a genuine interference with these seroreactions is not involved, we have, since 1955, titrated all listeric sera positive in the CFT which showed reactions with S. aureus antigen, in a comparative manner and effected reciprocal neutralization. A few examples of many are reproduced in Table 3, from which it is evident that the homologous listeria

titers cannot be caused to disappear either by sole neutralization of such type 1 sera with S. aureus antigen or enterococcal antigen or by combined absorption with both antigens.

Upon neutralization of such sera with listeria type 1 antigen sometimes all antibodies disappear, at times S. aureus antibody can be demonstrated even after removal of listeria antibody. The latter is clearly shown in sera of patients with chronic staphylococcal infections (Beeck, 3).

Thus a certain encroachment of listeriosis serology by thermostabile staphylococcal antigens does indeed exist. This may be eliminated only by the methodic steps listed here.

The significance of the thermostabile factors is still not clear, however. In this connection it should be remembered that Girard observed cross reactions between listeria hemolysin and staphylococcal toxin (9).

Taxonomically as well as practically important are furthermore, antigen associations between listeria and grampositive corynebacteria, especially the types pathogenic for man and animals. Potel (29a) and Osebold (personal correspondence) mention for instance agglutinations of Corynebacterium pyogenes and closely related strains in listeric sera. During precipitation tests with polysaccharide extracts of 10 strains of C. pyogenes var. hominis, 2 cultures of the gelatin-liquefying C. pyogenes as well as 10 additional corynebacterium types including 6 motile cultures probably belonging to C. helvolum and C. poinsettiae, we did not observe isolation by highly concentrated sera of all types of L. monocytogenes; but this fact in no way excludes the presence of other group components not belonging to the thermostabile polysaccharides. Finally, an observation by Lodenkamper (20) should be mentioned, who found cross reactions up to the titer limit in comparisons of acidophilus strains with his culture which we believe to be L. monocytogenes. Further persuation of this complex of questions also seems indicated.

While comparative tests of listeria and virus antigens are still lacking, something is known about the relation between listeria antigens on one hand and leptospira or toxoplasma antigens on the other:

Routine examination of a number of sera with a high agglutination titer against listeria antigen did not reveal signs of important titer interference during agglutination tests, CFT and agglutination-lysis tests with leptospira antigen as well as in reversed tests (32).

Analogous action was seen in comparisons of 861 sera, which were simultaneously examined for listeria and toxoplasma antibody, following pronouncements of suspicion of listeriosis and toxoplasmosis in the patients involved. Findings analyzed by Piekarski, Seeliger and Saathoff (27) are reproduced in part in Fig. 2 and 3.

The major part of the blood tests were either negative in both directions or only showed increased antibody levels against one of the two pathogens. The portion of blood specimens containing both toxoplasma and listeria antibody was always small. However, it exceeded theoretically expected values in the case

of toxoplasma antigen as well as listeria antigen. These mutual increases in titer values may possibly be a manifestation of double infection which is to be expected in view of quite similar epidemiological conditions. The percentage of probable double infections fluctuates in the tested, selected material between 0.6 and 2.9 of all specimens, or between 0.8 and 12.9 of "positively" reacting sera (listeria titer above 1:160, toxoplasma titer above 1:256).

In view of the possibility of influence of lipoid reactions on listeriosis serology in the CFT it should be mentioned that the Wassermann reaction was negative in almost all of our listeriosis-"positive" sera (32). Similarly it is certain that heterophilic antibodies are not stimulated by listeria antigen and that listeria seroreactions and the Paul-Bunnel test do not exert any influence on each other.

Thus, at the present stage of our knowledge and on the basis of extensive tests, a number of weighty arguments speak for the pathogen-specificity of antibody directed against listeria antigen found in human sera. —

The findings based on comparative antigen analytical tests are solidified even more by additional factors:

First of all it should be remembered that O and H antigens of listeria are different. Although at times only isolated O titers are found in human sera, O titers usually are accompanied by H titers. In view of the insufficient development of flagella and thus of H antigens at human body temperatures it is to be expected that in part of the infected persons the H titer will be far behind or will develop only imperfectly. On the other hand we know of observations in which first the H titer appeared and later the O titer.

Even if it is stipulated that a part of the O titers are caused by heterologous antibody, it appears rather improbable that corresponding H antibody should also be attributed to additional heterologous serum agglutinins.

The fact that these H agglutinations are not only directed against type 1H antigen but are effective also to the same extent against the serologically similar type 4H antigen, also speaks for a serologic specificity.

In addition, the frequency of antibody directed against O antigen of listeria type 1 is parallel to the preponderance of occurrence of type 1 in man and animal. The rarity of type 4O antibody in human sera corresponds to the small number of type 4 bacteriologic findings.

Contrariwise, Osebold and Sawyer (24a) in the USA, where type 4 predominates, found type 4-O agglutinins in low dilutions in a relatively high percentage of sera from healthy persons. As a partial corroboration of these findings Girard and Gavin (10) report from Canada that during examination of 100 sera from pregnant women in Dalhousie only 4% showed O agglutinins against listeria type 1. But type 4 antibody also could hardly be demonstrated; while 58% revealed at least partial H-1 titers up to a serum dilution of 1:256 and 16% gave vigorous reactions at this dilution against only 5% positive reactors with type 4-H antigen. A direct comparison with our results is not possible, because Girard's production of antigen and test technique differ somewhat from ours.

Considerable diagnostic puzzles are presented by isolated findings in which type 1 titers are higher in spite of assured type 4 infection.

Such a case, jointly examined by Girard, Gavin and by us (10), showed the following in the mother's serum: a type 1-O titer of 1:80, a type 1-H titer of 1:640 and a distinctly positive CFT at 1:5 with a negative type 4-O titer and a 4-H titer of 1:80. Type 4 CFT was negative. These titers are surprising for the reason that the strain isolated from the newborn definitely belonged to type 4b and that following absorption of the mother's serum the strain-specific antibody disappeared, but not so the 1-O and H agglutinins. The latter were easily exhausted by means of type 1 antigen.

Speculatively, a number of clarifications are possible, e.g. a secondary infection with type 4 without antibody formation following previous type 1 infection cured with residual titers and anamnestic antibody development, etc. —

The most important argument in favor of pathogen specificity of antibody effective against listeria antigen in agglutination tests as well as in the CFT consists of its conspicuous frequency precisely in connection with those pathological conditions which agree clinically with listeriosis, without being far-fetched.

This, for instance, is applicable to a certain group of central nervous disturbances, cerebral afflictions and postencephalitic conditions which are completely inexplicable in their etiology. Compared to similar aspects of known etiology this group reveals statistically significant increases in higher listeria titers, being especially vivid in the CFT. Lang (16) has reported on this after joint exploration with the author and has recently reaffirmed these findings published in 1954/55 by evaluating an even larger number of tests (17).

A second group of patients, as yet small in number, with elevated titers and partly typical titer progressions, is found among patients with Paul-Bunnell-negative general infections which accompany tumescence of the glands, liver and spleen, at times seen with encephalitic manifestations and peripheral mononucleosis.

The third group consists of cases of accumulated still births and miscarriages, collectively called "habitual abortions." This designation hides disturbances of known genesis (erythroblastosis due to Rh-incompatibility, toxoplasmosis) and some with unknown causes. Rost, Paul and Seeliger (30) found, in agreement with independent examinations at Prague (Patocka, Mencikova et al., 22) that a number of these unfortunate patients showed a distinctly positive reaction in the agglutination test and CFT with listeria antigen. At times such titers were obtained only at control tests in the 3rd and 5th month of renewed pregnancy. These titers could be lowered in part antibiotically with tetracyclin and subsequent supronal dispensations. Moreover, at the first attempt consistently applied therapy led to the full term and birth of a healthy child in an encouragingly high number of cases. So far, in dealing with 8 women with a total of 30 miscarriages, 6 cases resulted in striking success, among them a woman with 5 miscarriages who had specifically requested sterilization. (Fig. 5.)

Although Potel and Alex (1, 29b) have ascertained that after verified newborn listeriosis new pregnancies may occur without previous therapy, several cases are also found in the area of Halle who suffered a repeated miscarriage following previous listeriosis. We suspect that in listeriosis — analogous to other infectious diseases — a number of latent germ carriers remain which cannot as yet be isolated bacteriologically, in which case the normal course of gestation cannot be guaranteed due to the persistence of pathogens in the genitals.

In this connection a few short remarks should be made concerning serodiagnostic of listeriosis in animals, especially large animals. Doubtless infections in animals lead to the formation of antibody, partly even to the development of extremely high titers. However, the uncommonly frequent demonstration of antibody in the sera of healthy large and small animals without a history that might indicate clinical listeriosis makes evaluation so difficult that the research group around Graham resignedly refuted the importance of serolog as an aid in connection with infections in the animal kingdom (11). In the meantime Potel and Ehrenhardt (cited in 28), Dedie (5), Linsert (19), as well as Czech and Hungarian researchers (2, 4, 18 and others) have had similar experiences. Nevertheless it has been shown that serologically negative fowl show an extremely rapid increase in the number of positive reactors, even without clinical manifestations, at the occurrence of listeric infections, tending to support a supposition that the titers so frequently observed represent nothing but an expression of a latent, ubiquitous enzootic state and possibly even of occult immunization. Bastar et al. (2) and Lax (18) furthermore indicate the probability that a positive CFT may be feasible diagnostically. Recent publications by Osebold and others (24b) prove that despite the presence of basic titers experimental listeric infections (super infections?) evoke immune-biologic responses and measurable increases in antibody following previous immunization.

Without trying to usurp the place of veterinarians and veterinary bacteriologists, it seems that the serology and immune-biology of listeriosis in the animal kingdom promises many an epizootologically and diagnostically interesting disclosure.

I shall touch on the method of testing but briefly. We have recorded our experiences and results elsewhere (32). Whoever deals with this specialty of listeriosis research must realize that each alteration of method, be it in the selection of test strains or the production of antigens, must invariably lead to different results. We do not believe that the methods utilized by us are necessarily the best; but they can be duplicated and have shown entirely constant results in the course of 5 years. Above all they enabled us to compare new charges with proven antigens and to adjust them precisely. Unfortunately the changes in test methods frequently undertaken locally make any kind of comparison of research and results impossible in diverse places. We have repeatedly made astounding discoveries in checking some results and titers; at times the utilized antigens were worthless, sometimes there were titer differences by several grades of dilution. Part of the published findings are difficult to assess due to the fact that a number of research centers were furnished wrongly designated listeria type strains. In order to avoid such happenings we deposited our type strains four years ago with Prof. Dr. Hauduroy, Lausanne, from where they may be obtained. Unfortunately the strains are not always

prperly attended to. It has been proved also that cultures utilized for antigen production occasionally degenerate and thus lose their value for the evaluation of antigen-antibody reactions. Last but not least a titer determination has little sense, for the reason given, if the antigen comes from untyped strains, unless the strain isolated from the patient is available.

Frequently the question is raised as to which test process should be used. A generally valid answer may be given hereto in this sense that every type of serodiagnostic is improved by parallel utilization of several methods. First consideration goes to the agglutination reaction, the listeria-Widal, due to its unproblematic accomplishment. It is a useful aid when constantly reacting antigen preparations are available and when O or H antibody is analyzed separately. It has its limitations, however, for instance by the fact that its result is sometimes negative or shows low titers, while other reactions, e.g. the CFT, are strongly positive. We base this on bacteriologically verified findings. Whoever depends solely on the Listeria-Widal must expect to overlook some assured illnesses.

A supplemental method is offered by precipitation, which however has not proved itself to date, in contrast to initial favorable reports. It is not clear whether insufficient precipitation is due methodically to inadequate sensitivity of the test method or to actual absence of precipitating substances. The future will probably bring more favorable results. These, however, require more sensitive test methods, better antigens and repeated controls, especially in the first stadia of the disease.

Good results have been achieved with antigens produced according to specifications, as reported not only by Patocka and by us, but also by Mannweiler (21) who ascribes greater specificity to the complement fixation test, although it has been used by a small number of researchers only. Our own experiences and conclusions see in the CFT a valuable and indispensable supplement to the Widal reaction, without which valuable insight into immune-biologic processes would be lost to serology. As a rule, not only in listeriosis, deep and disseminated infections with involvement of the reticulo-endothelial system often, if not always, lead to a positive CFT, whose disappearance coupled with simultaneous improvement of clinical symptoms allows prognostically favorable conclusions. Frequently CFT titers are the only determinable manifestation of a latent infection. As an example the inapparent listeriosis of pregnancy may be mentioned, whose transition from the chronic to the acute statium dangerous to the fetus can be recognized as early as the 3rd and 4th month by a positive CFT. Certainly at times anamnestic reactions may be involved, but experience teaches that so-called unspecific titer elevations are much rarer than asserted heretofore.

Finally Potel's hemagglutination test (29a) should be mentioned, in which the hemosensitins of listeria, bound to erythrocytes, are utilized diagnostically. These detachable reaction products, in my opinion, are identical not with O antigen, but probably with a detachable polysaccharide fraction of a haptenal character. The titers therefore are not necessarily parallel to the O titers. Perhaps we are dealing here with the searched-for, sensitive, modified precipitin reaction as utilized in fungus antibody research. The method is not recommended as a routine, since comparative tests on a broad basis with simultaneous testing for agglutinating and complement fixing antibody are essential. To my knowledge

the last mentioned, decisively important control has not been accomplished. Then the degree of specificity and sensitivity must be tested. It must be remembered that the hemagglutination test, due to manifestation of trivial cross reactions between heterologous antigens and antibodies, shows such a high degree of sensitivity that its utilization in several areas of microbiology and serology is seriously endangered. In a serodiagnostic which is already weighed down with such an enormous quota of presently unfathomable "normal titers", a genuine contribution by hemagglutination can be expected only if it enables us to comprehend heretofore hidden parts of the antibody spectrum.

Whether there is such a thing as a "proving" listeriosis titer, we cannot say. Nevertheless CFT reactions with high dilutions of serum, i.e. with titers above 1:10, seem to indicate an existent, active infection. Low titers may have the same meaning, as is the case with residual titers following overcoming of the infection and clinical recovery. We have been checking some constant titers for over 3 years without seeing fluctuations of more than one dilution. Similar circumstances apply to agglutination titers, which are to be considered suspect from 1:320 up and possess a certain conclusiveness only at serum dilutions above 1:640. Thus we succeeded in diagnosing several cases serologically before the pathogen was demonstrated. On the other hand, titer values of 1:40 to 1:160, always missing in infants, but found in 70% of children up to 12 years and in about 30% of adults, may be the only sign of an existing infection. Thus evaluable data emerge only after repeated serum controls. This applies not only to listeriosis, but to many infectious diseases.

It is understandable that the practising physician will not be enthused by these facts. Perhaps more restraint in demands for serodiagnostic tests could be practised on the part of the clinic, since they are not only time-consuming but often unnecessarily burden the laboratory as much as the patient. In dealing with all acute, questionable infectious diseases an adequate amount of serum should be obtained as early as possible, it is then deep frozen; combined with an additional specimen after 7 to 10 days it will enable the laboratory to accomplish serodiagnostic, if the latter still proves to be necessary.

The situation is more difficult in dealing with suspected secondary conditions, since often only residual titers are demonstrable here, unless chronic-subacute processes are involved. While the residues do not show important titer changes following aimed chemotherapy, the last mentioned chronic cases of listeriosis are often accompanied by distinct and constant titer depressions after successful therapy, at times even by the disappearance of all previously positive seroreactions, just as relapse manifests itself by conspicuous titer peaks (Fig. 6).

Wherever possible, O and H agglutination tests and quantitative CFT should always be started parallelly; for only by comprehension of several parts of the antibody spectrum can a diagnostically evaluable and perhaps even prognostically significant insight into the immune-biological happenings be gained. Experience will show whether the combination of serologic methods utilized heretofore is adequate, or whether it can be advantageously supplemented by other tests. Next to the precipitation reactions the little used intracutaneous tests with correspondingly prepared antigens or antigen fractions deserve special consideration (12, 38). The opinion that this method is feasible in principle and even

promising of success, is supported by recent experiments with animals in the USA (7).

In closing it should be stressed again that the serodiagnostic method is always but an aid, which often is able to serve better in verifying the pathogenicity of a germ than in diagnosing a disease. The latter is usually possible only with typical titers.

The serodiagnostic of listeriosis can never replace or render superfluous the direct demonstration of the pathogen. On the contrary, where serologically positive or suspected factors appear, a search for the pathogen should be conducted with all available means. Research of listeriosis, especially its serodiagnostic, is still a young field and has only picked up speed in the last few years. While demanding a critical mode of observation, serologic and immunebiologic test methods promise many interesting epidemiological, epizootic and pathogenetic disclosures.

Table 1

Cross reactions between L. monocytogenes, serotypes 1, 2, 3, 4a and 4b, S. faecalis, strain L49, and S. aureus in the precipitin test with polysaccharide extracts (after Seeliger, Sulzbacher and Beeck)

Antigen	Antiserum						Normal serum control
	1-2	3	4a	4b	S.aureus (B3)	S.faecalis (L49)	
L.monocytogenes type 1	///	///	-	-	-	-	-
type 2	///	///	-	-	-	-	-
type 3	-	///	-	-	-	-	-
type 4a	-	-	///	//	-	-	-
type 4b	-	-	//	///	-	-	-
S.aureus (28 strains)	-	/(1)* //(7) ///(20)	-	-	/(3) //(6) ///(19)	-	-
S.faecalis (L49)	///	-	-	-	-	///	-

* Number of positively reacting strains.

Table 2

Frequency of positive CFT titers in the presence of L. monocytogenes, type 1 and 3, — as well as S. aureus antigen in 1,709 human sera.

Antigen		Positive CFT at serum dilution of 1:5 and higher
L. monocytogenes, serotype 1		264
of these:	serotype 1 alone	95 (36%)
	serotype 1 and serotype 3	95 (34.1%)
	serotype 1 and S. aureus	26 (9.8%)
	serotype 1, serotype 3 & S. aureus	53 (20.1%)
L. monocytogenes, seroype 3		359
of these:	serotype 3 alone	140 (39.1%)
	serotype 3 and serotype 1	90 (25.1%)
	serotype 3 and S. aureus	76 (21.1%)
	serotype 3, serotype 1 & S. aureus	53 (14.7%)
S. aureus		245
of these:	S. aureus alone	90 (36.8%)
	S. aureus and serotype 1	26 (10.6%)
	S. aureus and serotype 3	76 (31.0%)
	S. aureus, serotype 1 & serotype 3	53 (21.6%)

Table 3

Absorption tests with listeria-positive human sera in the quantitiative CFT.

Serum	absorbed by	Titer L. monocytogenes type 1	type 3	type 4b	S.aureus B3	S.faecalis L49	serum control
C622/55	-	10//	10//	-	5////	5//	-
	S.aureus B3	5///	5///	-	-	5//	-
	S.faec.L49	10//	5///	-	5///	-	-
and	S.aureus B3 S.faec.L49	5///	5///	-	-	-	-
C940/55	-	40//	10//	5///	5///	5///	-
	S.aureus B3	20//	5///	5///	-	5///	-
	S.faec.L49	20//	5///	-	5///	-	-
and	S.aureus B3 S.faec.L49	20//	5///	-	-	-	-

(continued next page)

Table 3 (continued from preceding page)

Serum	absorbed by	Titer L. monocytogenes type 1	type 3	type 4b	S.aureus B3	S.faecalis L49	serum control
C2235/55	-	40///	10///	5////	10///	40///	-
	S.aureus B3	40//	-	-	-	not done	
	S.faec.L49	40//	10///	5//	not done	-	-
Normal serum		-	-	-	-,	-	-

* reciprocal value of serum titers (50-percentual inhibitition of hemolysis equals //) (Translator's note: Table 3 is not starred)

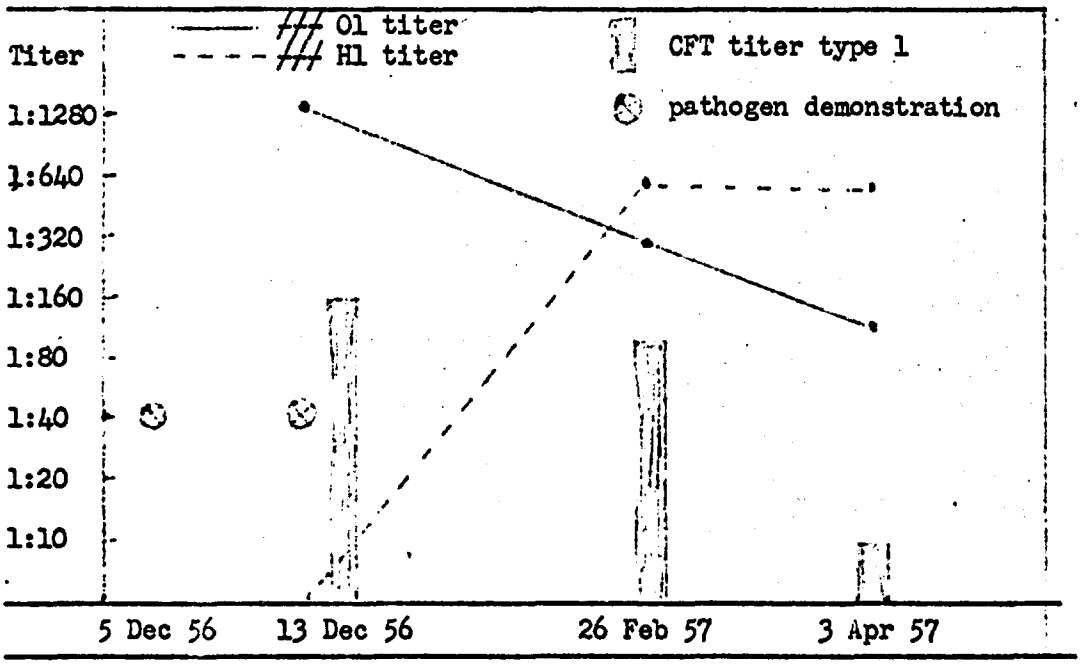

Fig. 1. Titer progression in a case of bacteriologically verified listeriosis of the cervical gland (Case E.B. — V-11 282/56, after Seeliger, Vogels, Teschner and Linzenmeier, 1956)

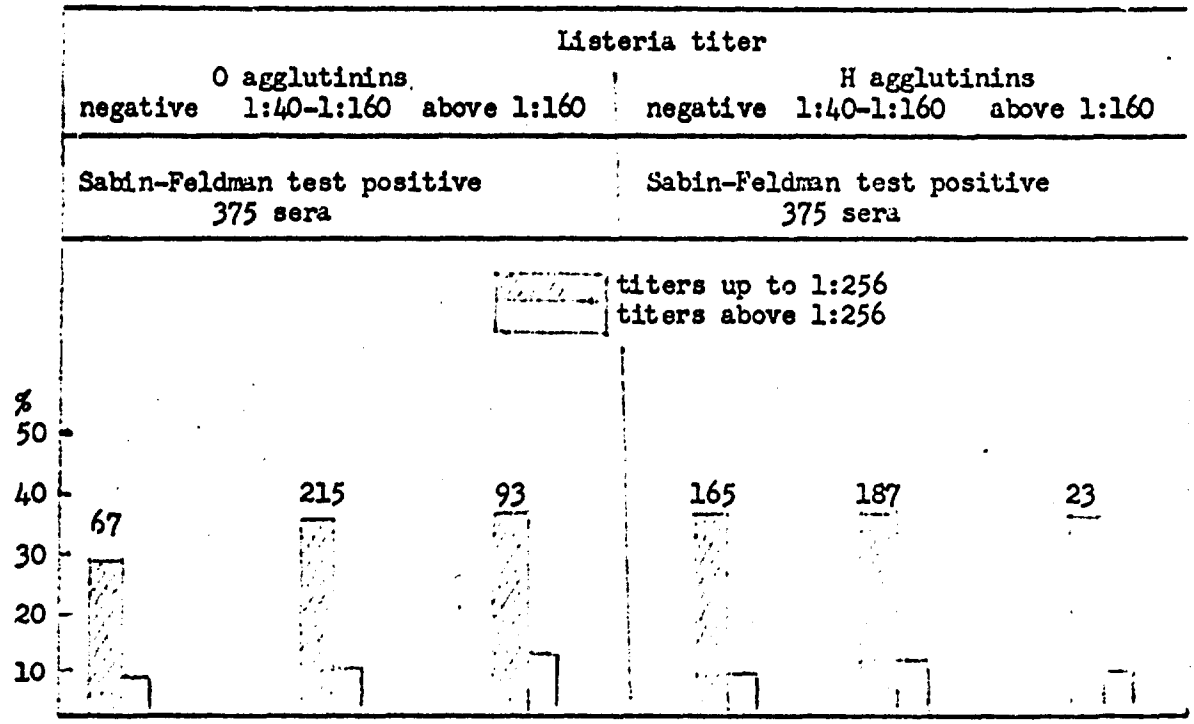

Fig. 2. Listeria O and H agglutinins in 375 Sabin-Feldman positive sera (after Piekarski, Seeliger and Saathoff, 1957)

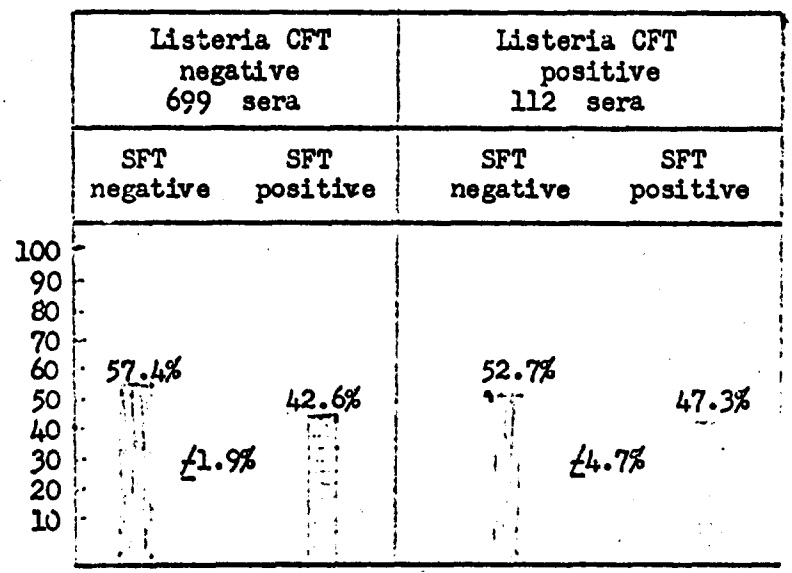

Fig. 3. Results of listeria CFT with 699 toxoplasma-negative sera (SFT) (after Piekarski, Seeliger and Saathoff, 1957)

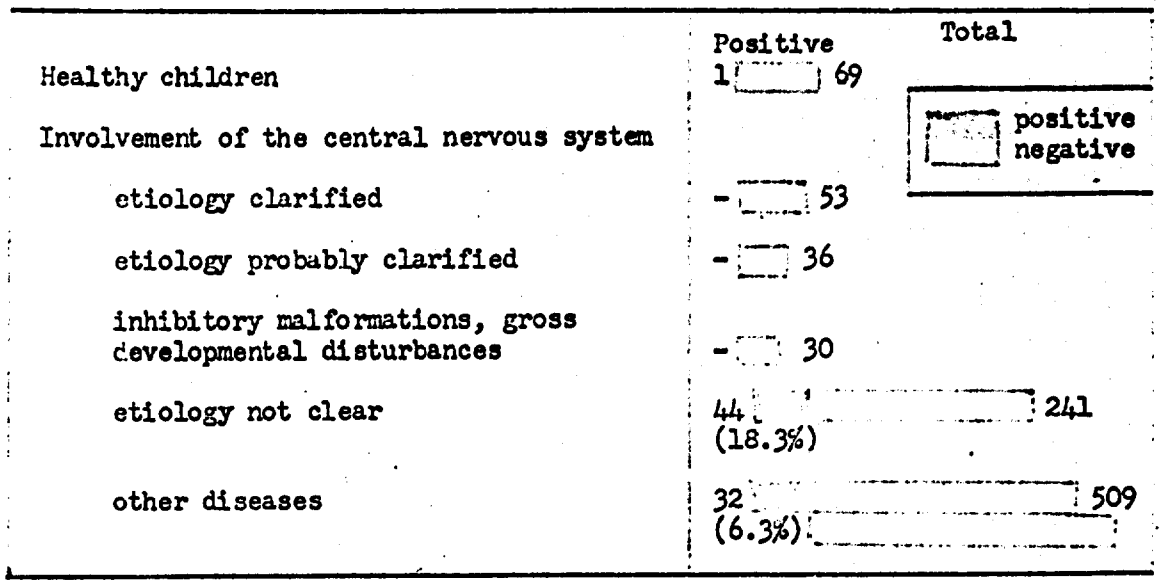

Fig. 4. Distribution of elevated listeria agglutination titers (1:320 and higher) and positive CFT with involvement of the central nervous system and other diseases in the childhood period (after Lang and Seeliger, 1957)

Case number	Without treatment							After treatment
Fr.E.Z.	+	+	+ °	+	+ °	+ °	+	♀
1240/55	+ °	+ °	+ °	+ °	+ °	+		♂
3137/53	+ °	+ °	+ °	+ °				♀
2206/55	+ °	+ °	+ °	+ °				♂
2108/49	+ °	+ °	+ °					
1273/56	+	+ °	+ °					
1259/56	+ °	+ °						♀
298/56	+							♂

Fig. 5. Therapeutic results for 8 patients with habitual abortion and serologically positive evidence of listeriosis (after Rost, Paul and Seeliger 1957) (° represents miscarriages)

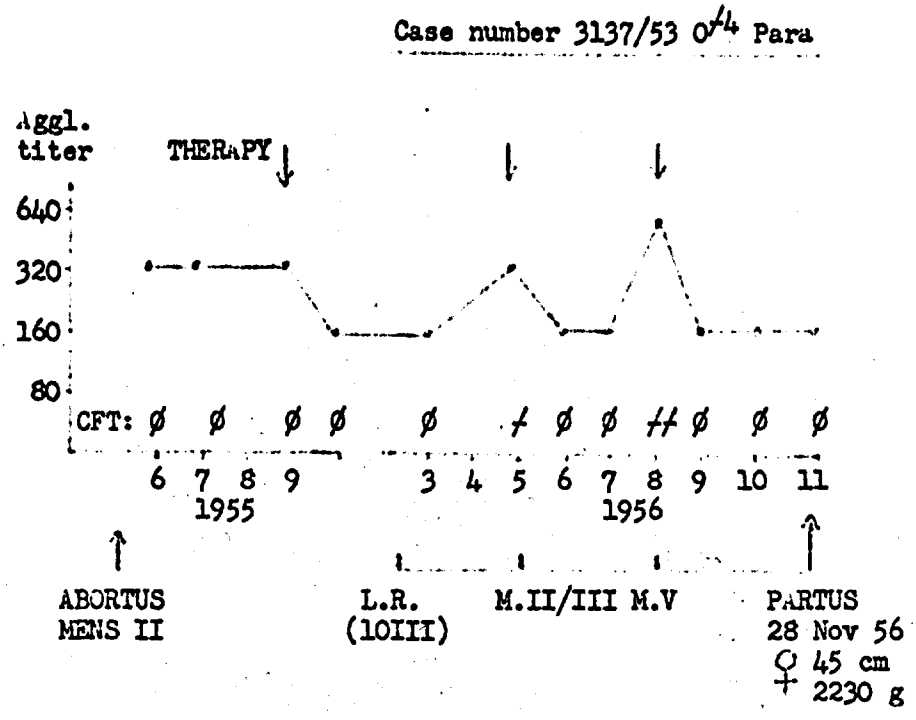

Fig. 6. Listeria titer curve at habitual abortion (after Rost, Paul and Seeliger, 1957)